Neuron-specific enolase correlates to laboratory markers of haemolysis in patients on long-term circulatory support†

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Abstract

OBJECTIVES: Neuron-specific enolase (NSE) is used as a diagnostic tool in neuropathies, cerebral diseases or traumata and for some tumours. Furthermore, it is also expressed by erythrocytes and platelets and has been linked to haemolysis ex vivo as a laboratory issue. Chronic haemolysis is frequently associated with mechanical circulatory support by ventricular assist device (VAD) or total artificial heart (TAH). Therefore, we compared NSE with indicators of haemolysis in VAD and TAH patients.

METHODS: We included 599 data sets of 97 patients who underwent VAD or TAH implantation. NSE, haptoglobin (HAPT), haemopexin (HPX), free haemoglobin (frHB), lactate dehydrogenase activity (LDH), platelet counts and total bilirubin (TBIL) in plasma were analysed. Further, all major cerebral events were assessed.

RESULTS: NSE correlated to frHB ($r_s = 0.553$) and to LDH ($r_s = 0.695$). An inverse correlation was found with HAPT ($r_s = -0.484$) and HPX ($r_s = -0.398$). Thirty-two patients suffered neurological events. Within the time frame of 1 day before to 4 days after a neurological event, correlations of NSE to HAPT ($r_s = -0.540$) and HPX ($r_s = -0.611$) in negative and to frHB ($r_s = 0.757$), LDH ($r_s = 0.862$) and TBIL ($r_s = 0.549$) in positive direction were established (all $P < 0.05$). Furthermore, haemolysis was graded into three groups for severe, moderate or no or only slight haemolysis. NSE values differed correspondingly between these groups ($P < 0.001$).

CONCLUSION: NSE correlates to laboratory parameters indicative of haemolysis in VAD and TAH patients. Our data suggest an influence of intravascular haemolysis on NSE. Therefore, the parameter should be used with caution when it is used to assess cerebral damage.

Keywords: Ventricular assist device • Neuron-specific enolase • Haemolysis • Cerebrovascular injury

INTRODUCTION

Neuron-specific enolase (NSE) has been discovered as a marker of neuronal and neuroendocrine cells and of neuronal differentiation. It is widely used as a diagnostic tool in neuropathies, cerebral diseases and trauma and as an oncological indicator for tumours of neuronal and neuroendocrine origin such as small-cell lung carcinoma and several gastrointestinal cancers [1, 2]. Later on, NSE was found to be expressed by erythrocytes and platelets, and the negative impact of haemolysis on the accuracy of NSE measurement in laboratory was emphasized [3, 4].

NSE has also been investigated in cardiac surgery.Increases were observed during and after operations using extracorporeal circulation. The contribution of concomitant neurological injury and of extravascular haemolysis to this finding is subject to discussion [5–7]. A comparable problem applies to the clinical course of NSE after implantation of long-term ventricular assist devices (VADs) [8, 9]. In these patients, chronic intravascular haemolysis frequently accompanies mechanical circulatory support [10]. Potapov et al. [8] advocated the use of NSE as a marker of brain injury in VAD patients. In contrast, Pfeifer et al. [9] suggested haemolysis to be a reason for significant increases of NSE. However, haemolysis was diagnosed only indirectly by decreases in platelet counts and haemoglobin levels in this study. Therefore, our analysis aims to compare NSE with laboratory markers of haemolysis, that is haptoglobin (HAPT), haemopexin (HPX), free haemoglobin (frHB) and lactate dehydrogenase activity (LDH). We hypothesize an association of these data in VAD and TAH patients. In addition, correlation of NSE to bilirubin was investigated.
MATERIALS AND METHODS

Patients

We included 599 data sets of 97 patients who underwent VAD or TAH implantation between March 2009 and June 2014: 76 with a HeartMate II® (Thoratec Corporation, Pleasanton, CA, USA), 14 with a biventricular PVAD® (BVAD Paracorporeal Ventricular Assist Device, Thoratec Corporation), 3 with a left-ventricular HeartWare® (HeartWare, Inc., Framingham, MA, USA) and 4 patients with a TAH (CardioWest®, SynCardia Systems, Inc., Tucson, AZ, USA).

Patients were analysed prior to and after VAD implantation for laboratory data. Further, all major cerebral events were assessed. These included strokes, cerebral bleeding events, prolonged reversible ischaemic neurological deficits and transitory ischaemic attacks. Non-specific occurrences such as delayed awakening without pathological correlates in CT scan were excluded.

This is a retrospective analysis of prospectively enclosed patients. All patients provided informed consent. The study was approved by the Ethics Committee of the University of Freiburg.

Surgical procedures

Implantation of the VADs or TAHs was performed using standard techniques. Anticoagulation for all systems was started with heparin with a target activated partial thromboplastin time of 60–80 s. It was changed to phenprocoumon after removal of the chest drains and sufficient oral ingestion. Target international normalized ratio was 2.0–3.0 for the HeartMate II and HeartWare, and 3.0–3.5 for the Thoratec BVAD and the CardioWest. Platelet aggregation was inhibited by acetylsalicylic acid.

Laboratory methods

NSE and HAPT were determined in serum by an immunological test (test and analyser: Roche Diagnostics, Mannheim, Germany). HPX was measured using an antibody-based nephelometric test (Siemens Healthcare Diagnostics, Eschborn, Germany; analyser: BN ProSpec, Siemens Healthcare Diagnostics). frHB in plasma was calculated after direct photometry in sodium citrate plasma at 380, 415 and 450 nm (Harboe method) on a spectral photometer (LS 500, Lange, Berlin, Germany). Lactate dehydrogenase activity (LDH) was measured enzymatically according to the International Federation of Clinical Chemistry and Laboratory Medicine method (Roche Diagnostics). Platelet counts were determined by employing standard laboratory procedures. Further, total bilirubin (TBIL) in serum was assessed according to the standard protocol (analyser: Cobas 8000 c702, Roche Diagnostics, Mannheim, Germany).

Statistics

The program PASW Statistics 21 (SPSS, Inc., Chicago, IL, USA) was used for all calculations. Correlations were tested using Spearman’s Rho ($r_s$). Correlation was considered weak for $|r_s| > 0.1$ to $0.3$, moderate for $|r_s| > 0.3$ to $0.5$ and strong for $|r_s| > 0.5$. The Mann-Whitney U-test was employed for comparisons between groups. The boxes of Figs 1B and 2 contain the middle 50% of the values (25th and 75th percentile), the median is marked. The whiskers indicate the upper and lower non-extreme values. Circles mark outliers (distance from the box between 1.5- and 3-fold length of the box), and asterisks mark extreme values (distance from the box more than 3-fold length of the box).

The three-step grading of haemolysis bases on Eyster et al. [11], Delanghe et al. [12] and Heilmann et al. [10]. Severe haemolysis is indicated by loss of both HAPT and HPX, moderate haemolysis by lacking HAPT but normal HPX and only slight or no haemolysis by normal HAPT.

RESULTS

A total of 599 data sets of 97 patients were analysed. Descriptive statistics and correlation of parameters of haemolysis and hepatic function to NSE are given in Table 1. NSE correlated strongly to frHB and to lactate dehydrogenase. A moderate inverse correlation was found to HAPT and HPX. Correlation was positively weak to TBIL.

Distribution of NSE in the peri- and postoperative course of VAD implantation (minimum 10 days before to maximum 844 days after surgery) is shown in Fig. 1A. Thirty-two patients suffered major neurological events with an onset of 3–241 days (mean 51 days ± standard deviation 63 days, median 22 days) after device implantation. Laboratory data derived within 1 day before to 4 days after the incident were available for 20 events. These values are marked in Fig. 1A. NSE and LDH of the data sets, which were associated with a neurological event, differed from those, which were not (Fig. 1B). However, an overlap of the groups was observed (Fig. 1A and B).

Table 2 presents the respective data. A strong correlation of NSE to HAPT and HPX in negative and to frHB, LDH and TBIL in positive direction was identified. It should be noted, that the median of HAPT is 5.0 mg/dl in this group. This is the lower limit of the test and the value was assigned arbitrarily even if HAPT was not detectable anymore. Hence, the distribution of values is not linear in this range and a correlation is more difficult to detect. This can result in a lower significance.

Furthermore, haemolysis was graded into three groups. Strong haemolysis is indicated by decreases of both HAPT and HPX whereas normal HPX at decreased HAPT is a sign of moderate haemolysis. Both values are normal, when no or only slight haemolysis is present. NSE values differed between these groups as illustrated in Fig. 2.

DISCUSSION

The association of NSE with parameters of haemolysis was analysed in patients with implantation of a VAD or TAH. We observed a good correlation of NSE to markers of haemolysis, that is, HAPT, HPX, frHB and LDH. This was also true for samples, which were obtained shortly before or after a major neurological event.

Haemolysis has been shown to occur frequently in patients on VAD support [10]. Of note, much higher thresholds for normal frHB in plasma are applied to VAD patients than to other ones. Haemolysis on VAD support is diagnosed at levels of above 15 to even 40 mg/dl [13–15] compared with 5 mg/dl for normal persons. An increase of haemolysis can indicate pump thrombosis, which can be a precedent of cerebral embolic events and predict adverse outcome [13, 16, 17]. A recent guideline recommends periodical routine screening of LDH and frHB in addition to haemoglobin or haematocrit to check for haemolysis [15]. However, measurements...
of LDH and frHB are prone to preanalytic in-sample haemolysis and to interference with bilirubinaemia, lipidaemia and other factors. In VAD patients, LDH has been suggested to indicate haemolysis more reliably than frHB [17].

HAPT is employed as an early indicator of haemolysis in VAD patients [16]. It is an acute-phase protein, which can achieve values far above normal under inflammatory conditions. However, such processes do not seem to influence the plasma level in haemolytic patients [18]. This could be explained by the effective depletion of HAPT when any frHB is present because it is the 'first line of defence' against frHB [19]. For these reasons, neither frHB nor HAPT is an entirely dependable indicator for the severity of haemolysis. The 'second-line scavenger' HPX can be helpful for detection and monitoring of moderate haemolysis with exhausted HAPT and modestly increased frHB [12]. Assays for HAPT and HPX do not react to in-sample haemolysis.

Abnormal low levels of HAPT and HPX without haemolysis can result from reduced synthesis due to liver function impairment [12, 18]. Diagnostics of haemolysis remains difficult in patients with hepatic insufficiency since high T Bil can interfere with frHB analysis in several laboratory assays. Of note, independency of NSE and bilirubin values was reported in a previous study [20].

An increase of NSE was found immediately after VAD implantation with return to normal on the first postoperative day in a study on 15 patients [8]. In contrast, a broad spread of values was observed in our analysis, even in patients who never had a neurological event in the postoperative course.

Considering the strong association of NSE with indicators of haemolysis in our overall study population and in the population with neurological events, an influence of intravascular haemolysis on NSE is likely. The difference for NSE between data sets with or without association to a neurological event (yes) is similar to that for LDH, an established marker of haemolysis. In our opinion, this finding does not support the usability of NSE for the assessment of neurological injuries in VAD patients, but the association of NSE with haemolysis.

In patients with cerebral injury, NSE has been shown to correlate with findings in CT scans and with clinical parameters. However, expression levels differ also between types of injury. Overall, the use of NSE as a sole and independent parameter is

Figure 1: (A) Distribution of NSE in the course of VAD implantation. Green dots indicate values, which were derived at 1 day before to 4 days after a major neurological event. (B) Comparison of NSE (left panel) and LDH (right panel) for data sets out of (no) and within 1 day before to 4 days after a neurological event (yes). The ordinate for LDH is exponentially scaled for better resolution of the lower value range. Dashed lines represent the upper normal limits for NSE and LDH, respectively. The boxes contain the middle 50% of the values, the median is marked. The whiskers indicate the upper and lower non-extreme values. Circles and asterisks designate outliers and extreme values, respectively. NSE: neuron-specific enolase; VAD: ventricular assist device; LDH: lactate dehydrogenase.
not advocated but it is considered a valuable tool when integrated in the usual diagnostic process [21, 22]. NSE peaks 1–3 days after the neurological event (reviewed in [23]). We chose this time frame for analysis of patients with cerebral events accordingly for this study with 1 additional day before symptoms occurred to account for any delay in their appearance. However, our data are not sufficient to prove an indicative or even a predictive value of NSE for cerebral events. The laboratory data were collected according to our perioperative schedule. Therefore, data following neurological events are incomplete. As a further limitation of our study, we are not able to exclude the influence of activated platelets and macrophages. These cells have been suggested as sources for elevated NSE levels in some inflammatory diseases [24, 25].

In conclusion, our data indicate that NSE correlates well to laboratory parameters indicative of haemolysis in patients with long-term mechanical circulatory support. We hypothesize that an increased NSE due to the damage of erythrocytes could cause false-positive results in the assessment of cerebral injuries. We suggest that a possible influence of intravascular haemolysis on NSE should be considered when this parameter is used to evaluate cerebral damage.

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Table 1: Descriptive statistics of laboratory parameters and correlation with NSE for all data sets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Median</th>
<th>Range</th>
<th>Interquartile range</th>
<th>Not normal, n (%)</th>
<th>( r_s )</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSE</td>
<td>&lt;17 µg/l</td>
<td>599</td>
<td>29.9</td>
<td>4.1–197.2</td>
<td>21.0</td>
<td>529 (88%)</td>
</tr>
<tr>
<td>HAPT</td>
<td>&gt;30 mg/dl</td>
<td>592</td>
<td>7.0</td>
<td>5–612</td>
<td>83</td>
<td>362 (61%)</td>
</tr>
<tr>
<td>HPX</td>
<td>&gt;0.5 g/l</td>
<td>529</td>
<td>0.6</td>
<td>0.05–2.56</td>
<td>0.5</td>
<td>210 (40%)</td>
</tr>
<tr>
<td>FrHB</td>
<td>&lt;5 mg/dl</td>
<td>398</td>
<td>6.0</td>
<td>0.1–117</td>
<td>11.1</td>
<td>223 (56%)</td>
</tr>
<tr>
<td>LDH</td>
<td>&lt;225 U/l</td>
<td>588</td>
<td>417</td>
<td>87–7520</td>
<td>291</td>
<td>559 (95%)</td>
</tr>
<tr>
<td>TBIL</td>
<td>&lt;1.4 mg/dl</td>
<td>583</td>
<td>1.4</td>
<td>0.2–38.9</td>
<td>2.4</td>
<td>300 (51%)</td>
</tr>
</tbody>
</table>

NSE: neuron-specific enolase; HAPT: haptoglobin; HPX: haemopexin; FrHB: free haemoglobin; LDH: lactate dehydrogenase; TBIL: total bilirubin in serum; \( r_s \): correlation to NSE, n.a.: not applicable, **P < 0.01.

Figure 2: Comparison of NSE for data sets indicating severe (HAPT and HPX path), moderate (HAPT path, HPX normal) or no or only slight haemolysis (HAPT normal). For explanation of graphic elements, see legend to Fig. 1B. NSE: neuron-specific enolase; HAPT: haptoglobin; HPX: haemopexin.

Table 2: Descriptive statistics of laboratory parameters and correlation with NSE for data assessed 1 day before to 4 days after a neurological event

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Median</th>
<th>Range</th>
<th>Interquartile range</th>
<th>Not normal, n (%)</th>
<th>( r_s )</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSE</td>
<td>20</td>
<td>41.7</td>
<td>13.3–197.2</td>
<td>35.7</td>
<td>19 (95%)</td>
<td>n.a.</td>
</tr>
<tr>
<td>HAPT</td>
<td>19</td>
<td>5.0</td>
<td>5–238</td>
<td>47</td>
<td>14 (74%)</td>
<td>-0.540*</td>
</tr>
<tr>
<td>HPX</td>
<td>19</td>
<td>0.3</td>
<td>0.05–1.05</td>
<td>0.6</td>
<td>10 (53%)</td>
<td>-0.611**</td>
</tr>
<tr>
<td>FrHB</td>
<td>13</td>
<td>18.0</td>
<td>2.2–116</td>
<td>50.4</td>
<td>11 (85%)</td>
<td>0.747**</td>
</tr>
<tr>
<td>LDH</td>
<td>18</td>
<td>883</td>
<td>320–6260</td>
<td>1459</td>
<td>18 (100%)</td>
<td>0.862**</td>
</tr>
<tr>
<td>TBIL</td>
<td>20</td>
<td>2.9</td>
<td>0.6–22.9</td>
<td>7.85</td>
<td>15 (75%)</td>
<td>0.549*</td>
</tr>
</tbody>
</table>

For abbreviations and normal values, see Table 1. n.a.: not applicable, *P < 0.05, **P < 0.01.
Conflict of interest: Ulrich Geisen receives research funding by Roche Diagnostics GmbH, Germany, and Roche Diagnostics International AG, Switzerland. Christoph Benk, Friedhelm Beyersdorf and Georg Trummer hold shares in ResuSciTec Ltd, Freiburg im Breisgau, Germany.

REFERENCES


[13] Breisgau, Germany. International AG, Switzerland. Christoph Benk, Friedhelm Beyersdorf by Roche Diagnostics GmbH, Germany, and Roche Diagnostics Con


APPENDIX. CONFERENCE DISCUSSION

Dr H Najm (Riyadh, Saudi Arabia): A neurological event is the most devastating complication after assist-device implantation, and this paper correlates NSE to markers of intravascular haemolysis in an attempt to predict pump thrombosis, thus, neurological events.

I have to say that the manuscript is still missing some important data, which should be included in the final published manuscript, such as patient demographics, indications for insertion of the pump, as well as the management of anticoagulation and the level of anticoagulation at the time of the event; this would give a complete picture of what is happening with the patients at the time of the neurological event. Also the data which has been mentioned in the manuscript includes only 20 patients out of the 30 patients who had the events, and that is 30%. That is 30% less than the data that is included, and the conclusions may differ if you include the entire patient population in the data analysis. I would suggest that the data should be analysed in a multivariate analysis including a model putting in the NSE, as well as haemolysis factors so you would look at the predictability of this test versus the others for the neurological events. I have three straightforward questions to you.

First, since you have included in your series a combination of axial, centrifugal and pulsatile flow pumps, have you looked at the variation of the levels of the tests you have done in comparison with the different pumps used and included in this series?

Dr Heilmann: First of all, the goal was not to use the NSE for assessment of cerebral injuries. We do not do this because, according to our long-time experience, it is not reliable. We also did no regular testing at the time-point of bleedings. So these samples are taken by chance, so to speak, and we only want to raise awareness that the NSE correlates to haemolysis and, therefore is probably not reliable for assessing cerebral damage in patients on VAD or TAH.

Regarding the numbers, the group sizes are really small for the three smaller groups and we therefore refrained from analysing the devices. However, we have shown before that the TAH and biventricular VAD have higher grades of haemolysis, so we just want to point out the haemolysis, and it didn’t matter for us which device it was.

Dr Najm: My second question is, did you actually look at pump thrombosis itself? Did the haemolysis actually correspond to pump thrombosis in any of the patients you have seen or when you went back to them?

Dr Heilmann: It did not correspond.

Dr Najm: And what do you think about the applicability of this test in the future in clinical practice?

Dr Heilmann: We think you can use NSE as a good parameter for haemolysis but not for cerebral damage, at least in these patients.